

STUDIES ON LEUKOCYTOSIS *

I. HOURLY LEUKOCYTE VARIATIONS OF NORMAL RABBITS

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Few studies have been reported of the variations of individual rabbits whose leukocytes have been counted at short intervals during the day. The purpose of this report is to present results of total and differential leukocyte examinations of 14 normal rabbits, studied at hourly intervals.

In the report by Sabin, Cunningham, Doan and Kindwall¹ of "the normal rhythm of the white blood cells" in human subjects, brief mention is made that experiments on the blood of rabbits, similar to the reported experiments on humans, showed "the same general rhythms." They published one chart of one rabbit, studied at consecutive 30 minute intervals from 11 a.m. to 1:15 p.m. This chart shows considerable variation in total leukocyte counts and numbers of polymorphonuclear neutrophilic leukocytes (amphophils) and lymphocytes. Bushnell and Bangs² tabulated the total and differential leukocyte counts of one rabbit, examined at consecutive 45 to 60 minute intervals during the day on five occasions over a period of 43 days. On each day no determinations were made during a 2-hour interval around noon. Under these conditions, considerable daily variation occurred in the leukocyte counts of this rabbit. Cheng³ presented the total leukocyte counts of two rabbits, studied at consecutive 15 to 30 minute intervals. One rabbit was examined during the afternoon of one day and again during the morning of the following day. The other rabbit was studied during the afternoon of one day and again, 2 days later, from late morning through the afternoon. Cheng stated that the total counts of both rabbits tended to show

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similar variations, consisting of two rises, one at 10 a.m. to 12 noon, and the other at 3 to 4 p.m. There were also sudden variations of about 2500 leukocytes at short intervals.

In these reports, no mention is made of the number of 1 sq. mm. unit areas which were examined in determining the total leukocyte counts of each rabbit. Sabin and co-workers¹ did not state the number of leukocytes which they studied in making the differential examinations of their rabbit. Bushnell and Bangs² counted and classified approximately 200 leukocytes in making their differential determinations.

Despite the scarcity of reports of daily leukocyte variations of individual rabbits, a voluminous literature has accumulated concerning other aspects of leukocyte variations of rabbits. This literature has been summarized by Cheng³ and Garrey and Bryan.⁴ An analysis of errors in leukocyte counting has been presented by Bryan, Chastain and Garrey.⁵ Anyone working with variations in leukocyte counts is impressed with the numerous possible sources of error in the determinations, involving all procedures from selection of subjects to the actual counting of the leukocytes.

A large number of cells should be counted in making total and differential leukocyte determinations if significant results are to be obtained. The importance of counting enough cells in making total leukocyte counts is evident from studies of leukocyte variations in normal human subjects by Sabin and co-workers¹ and Ponder, Saslow and Schweizer.⁶ Sabin *et al.*, counting the cells on "the same side of a counting chamber" in five of six total leukocyte determinations, described hourly rhythmical leukocyte variations. Ponder and co-workers obtained similar apparent hourly rhythms when the cells on "one side of one counting chamber" were counted, but noted that these rhythms tended to disappear and much less fluctuation in counts occurred when the cells on "both sides of 2 counting chambers" were counted. It seems advisable to record the number of cells enumerated in making total leukocyte counts in terms of a standard, such as "unit areas of one square millimeter each," recognizing that one side of a certified counting chamber contains nine such unit areas.⁵

After every attempt has been made to eliminate possible sources of error in the determinations, irregular variations may

still occur in leukocyte counts of rabbits made at short intervals during the day.^{4,7} Observations of human leukocyte variations indicate that fairly frequently the total leukocyte count increases moderately in the afternoon, so that the average of the afternoon counts may be higher than the average of the morning counts.^{1,6,8} In many instances this afternoon increase in human subjects is apparently due mainly to an absolute increase in the number of polymorphonuclear neutrophilic leukocytes.^{1,8,9}

The following observations of hourly leukocyte variations of 14 normal rabbits are presented. These studies were made in connection with experiments on leukocytosis to be reported subsequently.

PROCEDURE

Fourteen adult New Zealand white and mixed rabbits, weighing approximately 2.5 to 3.2 Kg. each, were studied during the period from May 24, 1939 to January 23, 1940. Each animal was kept in the same individual cage throughout this period and for several months prior to the day of counting. During this time the rabbits were removed from their cages at least once daily and appeared adjusted to handling. The temperature of the cages and counting rooms was maintained at approximately 20 to 25° C. One animal (B) was counted for two series of observations. No differences were noted between the counts of males and females, and both sexes were used. Rabbits with ear canker, snuffles, or any other abnormality, including abnormal eating or activity, were discarded. As the incidence of coccidia infestation appears worthy of consideration in reports dealing with leukocyte variations of the rabbit,¹⁰ mention should be made that the incidence of coccidiosis has been low in autopsied animals of similar breeds kept under identical conditions.

Food was placed in each rabbit cage once daily at approximately the same time (9 to 11 a.m.). The animals ate slowly over a period of 3 to 4 hours. The diet consisted of a complete ration chow, carrots and water. The rabbit chow was guaranteed by the manufacturer to have the following analysis: crude protein not less than 13.5 per cent, crude fat not less than 2.5 per cent, carbohydrate in crude fiber not less than 16 per cent, and carbohydrate in nitrogen-free extract not less than 47 per cent. It was

guaranteed to contain the following ingredients: wheat germ, soy bean oil meal, cut alfalfa, crushed oats, crushed barley, wheat middlings (standard), corn germ meal, crushed corn, molasses, calcium carbonate (limestone) 0.5 per cent, and iodized salt 0.5 per cent. No changes were made in feeding on the days of counting.

Leukocyte counts were made during the same general period of the day, beginning at approximately 9 to 9:45 a.m., with hourly determinations through 3:45 to 4 p.m. All total and differential examinations were made by one individual. Special consideration was given to the handling of the rabbits and all other procedures dealing with the collection and examination of blood samples to ensure the utmost accuracy of the results.

Blood was obtained from the lateral marginal vein of the left ear, punctured by a Hagedorn needle. Freely flowing drops of blood were considered essential. The same certified pipette was used for an animal throughout a day of counting. The diluting fluid was 1 per cent acetic acid, freshly prepared and filtered. Pipettes were shaken by hand for 3 minutes and discharged into duplicate counting chambers after the first three or four drops had been discarded. Certified Zeiss chambers with the Neubauer ruling and polished glass shoulders were used, and coverglasses were carefully adjusted so that proper depth of the counting chambers was obtained. Approximately 10 minutes was allowed for settling of the leukocytes.

The cells in the four large corner squares of both sides of two chambers were counted, so that 800 cells were actually enumerated when the total number of leukocytes was 10,000 per cu. mm. Thus in each case sixteen unit areas of 1 sq. mm. each were examined.

At least three blood smears were prepared for each hourly count. Smears were made on slides which had been freshly cleaned with acid alcohol and polished with a clean dry towel. Films were stained with Wright's stain and studied with the oil immersion objective, at a magnification of $\times 970$. In each case, 300 leukocytes were counted, essentially the same areas being examined on each slide.

Leukocytes were classified into five general groups: polymorphonuclear neutrophils (amphophils), lymphocytes, large

mononuclear cells (monocytes), eosinophils, and basophils. In the present study, neutrophils (amphophils) were not subdivided into classes according to the appearance of the nuclear mass. The number of unclassified cells was less than 0.5 per cent in each case. Confusion was only occasionally encountered between lymphocytes and large mononuclear cells.

Total leukocyte counts were recorded to the nearest hundred. Differential percentages were adjusted to the nearest integer. Averages of the hourly total and absolute leukocyte counts and differential percentages of all rabbits were calculated by averaging counts made at seven consecutive hourly periods (from 9:45 to 10:05 a.m. through 3:45 to 4:05 p.m.). Averages of all counts of the day for each rabbit were also obtained. Morning and afternoon averages for individual rabbits were obtained by averaging all determinations during each respective period. In Table VI, differences between morning and afternoon averages are expressed as percentages of the morning average in each case.

TABLE I
Differential Leukocyte Examinations of 14 Normal Rabbits

Rabbit letter	Times of feeding and counts	Total leukocytes per cu. mm.	Differential percentages (300 leukocytes)				
			Neutrophils (amphophils)	Lymphocytes	Large mononuclear cells	Eosinophils	Basophils
A	9:15 a.m.*						
	9:25 a.m.	7000	22	71	2	0	6
	10:25 a.m.	8800	21	71	1	1	6
	11:25 a.m.	7700	34	60	2	1	3
	12:25 p.m.	6600	32	60	3	1	5
	1:25 p.m.	11900	34	61	1	0	4
	2:25 p.m.	12200	33	65	1	1	1
	3:25 p.m.	14900	38	57	1	1	3
B	9:15 a.m.*						
	10:00 a.m.	7000	13	81	2	0	3
	11:00 a.m.	5900	14	80	2	1	3
	12:00 noon	5100	13	82	2	0	3
	1:00 p.m.	6500	25	65	2	3	5
	2:00 p.m.	7000	20	74	1	0	4
	3:00 p.m.	7900	49	45	2	1	4
	11:00 a.m.*						
B	9:45 a.m.	6000	10	86	2	0	2
	10:45 a.m.	5600	16	81	1	0	2
	11:45 a.m.	5800	23	73	0	0	3
	12:45 p.m.	9500	46	51	1	0	2
	1:45 p.m.	7100	33	62	1	1	3
	2:45 p.m.	6700	56	39	0	0	4
	3:45 p.m.	6700	52	43	1	0	4

* Indicates time of feeding.

TABLE I—(Continued)

Rabbit letter	Times of feeding and counts	Total leukocytes per cu. mm.	Differential percentages (300 leukocytes)				
			Neutrophils (amphophils)	Lymphocytes	Large mononuclear cells	Eosinophils	Basophils
C	9:30 a.m.*						
	10:00 a.m.	11800	34	56	2	1	8
	11:00 a.m.	8600	27	64	1	2	5
	12:00 noon	7700	36	54	2	1	6
	1:00 p.m.	8600	45	46	1	1	8
	2:00 p.m.	11500	41	43	1	0	15
	3:00 p.m.	7200	39	47	1	1	12
D	9:40 a.m.*						
	9:00 a.m.	7300	11	86	1	0	2
	10:00 a.m.	6900	23	70	1	1	5
	11:00 a.m.	7500	22	71	2	1	4
	12:00 noon	10400	33	64	1	0	2
	1:00 p.m.	7700	39	55	2	0	4
	2:00 p.m.	8400	38	56	2	1	3
E	10:00 a.m.*						
	9:55 a.m.	5500	41	55	1	1	2
	10:55 a.m.	5600	35	60	1	1	3
	11:55 a.m.	6300	31	62	3	1	4
	12:55 p.m.	11100	35	62	0	2	2
	1:55 p.m.	4600	34	60	1	2	3
	2:55 p.m.	6300	49	46	1	0	4
F	3:55 p.m.	8200	55	40	0	0	5
	9:30 a.m.*						
	10:05 a.m.	10600	23	72	1	1	2
	11:05 a.m.	7900	23	75	1	0	1
	12:05 p.m.	9700	21	72	1	2	4
	1:05 p.m.	8900	17	80	1	0	2
	2:05 p.m.	10200	24	72	1	1	2
G	3:05 p.m.	11000	29	64	1	0	6
	4:05 p.m.	10800	37	56	1	1	4
	10:30 a.m.*						
	9:45 a.m.	11300	24	70	4	0	2
	10:45 a.m.	10200	36	58	3	0	2
	11:45 a.m.	9600	33	56	5	0	6
	12:45 p.m.	8900	31	60	3	0	6
H	1:45 p.m.	10600	37	55	3	1	5
	2:45 p.m.	8300	32	63	3	0	3
	3:45 p.m.	8900	29	63	2	0	6
	10:00 a.m.*						
	9:45 a.m.	8200	41	53	2	0	4
	10:45 a.m.	9000	39	56	1	1	4
	11:45 a.m.	9800	39	55	1	0	5
	12:45 p.m.	8300	39	53	1	1	6
	1:45 p.m.	7900	45	49	0	1	5
	2:45 p.m.	9200	50	42	0	1	7
	3:45 p.m.	8300	47	50	0	0	3

* Indicates time of feeding.

TABLE I—(Continued)

Rabbit letter	Times of feeding and counts	Total leukocytes per cu. mm.	Differential percentages (300 leukocytes)				
			Neutrophils (amphophils)	Lymphocytes	Large mononuclear cells	Eosinophils	Basophils
I	10:00 a.m.*						
	9:45 a.m.	12200	11	87	1	0	1
	10:45 a.m.	10600	11	84	2	0	3
	11:45 a.m.	10100	14	82	2	0	3
	12:45 p.m.	11600	12	76	3	1	8
	1:45 p.m.	9000	18	72	2	1	7
	2:45 p.m.	10500	18	76	1	2	4
J	3:45 p.m.	10500	15	80	1	0	3
	9:30 a.m.*						
	10:45 a.m.	9300	21	69	2	0	7
	11:45 a.m.	9800	27	65	2	2	5
	12:45 p.m.	11700	28	63	2	1	5
	1:45 p.m.	11600	33	59	1	2	6
	2:45 p.m.	10000	34	57	2	1	7
K	3:45 p.m.	9300	37	54	3	0	6
	11:00 a.m.*						
	9:45 a.m.	12500	21	75	2	1	1
	10:45 a.m.	12100	21	71	3	0	5
	11:45 a.m.	15700	25	68	1	0	5
	12:45 p.m.	14200	34	60	2	0	4
	1:45 p.m.	10200	43	53	1	1	2
L	2:45 p.m.	12600	40	53	1	1	5
	3:45 p.m.	10900	29	65	1	0	5
	11:00 a.m.*						
	9:55 a.m.	15800	38	56	1	1	4
	10:55 a.m.	16200	39	57	3	0	1
	11:55 a.m.	14300	45	45	3	1	6
	12:55 p.m.	16200	48	47	2	1	3
M	1:55 p.m.	13700	40	57	1	0	1
	2:55 p.m.	14300	44	52	1	0	3
	3:55 p.m.	14600	44	53	1	0	2
	10:30 a.m.*						
	9:45 a.m.	9100	5	90	0	0	4
	10:45 a.m.	15000	14	83	1	0	2
	11:45 a.m.	13800	15	81	0	0	4
N	12:45 p.m.	9000	30	66	1	0	3
	1:45 p.m.	9800	36	58	1	1	4
	2:45 p.m.	10500	25	70	1	1	4
	3:45 p.m.	10900	21	76	0	0	2
	10:30 a.m.*						
	9:55 a.m.	5200	13	70	3	3	11
	10:55 a.m.	5600	27	65	3	0	5
	11:55 a.m.	6600	38	53	1	1	7
	12:55 p.m.	5700	44	47	1	3	4
	1:55 p.m.	6700	51	43	0	1	4
	2:55 p.m.	6400	38	50	1	2	9
	3:55 p.m.	8700	48	46	1	1	4

* Indicates time of feeding.

RESULTS

Results are presented in tabular form. Table I shows for each experiment the times of counts, the times that food was placed in the rabbit cages, total numbers of leukocytes per cu. mm. and differential percentages of neutrophils (amphophils), lymphocytes, large mononuclear cells, eosinophils, and basophils. As may be seen, there is considerable daily variation in different experiments and the time of placing food in the cages seems to bear no direct relationship to these variations. Variations in averages of all counts of the day of each rabbit, presented in Table II, likewise are of considerable magnitude. Repeated leuko-

TABLE II
Averages of Total Leukocyte Counts, Differential Percentages and Absolute Numbers of Leukocytes for All Counts of the Day of 14 Normal Rabbits

Rabbit letter	Average total leukocytes per cu. mm.	Average differential percentages and absolute numbers of leukocytes per cu. mm.			
		Neutrophils (amphophils)		Lymphocytes	
		Per cent	Absolute number	Per cent	Absolute number
A	9871	30	3122	63	6211
B	6566	22	1549	71	4579
B	6771	33	2397	62	4095
C	9233	37	3416	52	4759
D	8033	27	2277	67	5338
E	6800	40	2744	55	3730
F	9871	25	2491	70	6870
G	9685	31	3067	61	5886
H	8671	43	3712	51	4437
I	10642	14	1485	79	8496
J	10283	30	3090	61	6287
K	12600	30	3786	63	8047
L	15014	42	6389	52	7873
M	11285	21	2291	75	8524
N	6414	37	2461	53	3362
Average	9449	31	2952	62	5900

cyte examinations of 3 animals at approximately the same time on different days, prior or subsequent to the day of hourly counting, tend to show that, in general, each rabbit seems to have its own characteristic leukocyte picture and variations appear to be decreased under such conditions (Table III). Repeated leukocyte examinations of the other rabbits showed similar results.

Averages of the hourly total and absolute leukocyte counts and differential percentages of all 14 rabbits (Table IV) fail to reflect

TABLE III
*Repeated Leukocyte Examinations of 3 Normal Rabbits at Approximately
the Same Time of Day on Different Days*

Rabbit letter	Time	Total leukocytes per cu. mm.	Neutrophils (amphophils)		Lymphocytes	
			Per cent	Absolute number	Per cent	Absolute number
B	10:00 a.m.	9500	11	1045	83	7885
	10:30 a.m.	7200	11	792	84	6048
	10:20 a.m.	7000	5	350	88	6160
	10:00 a.m.	7000	13	910	81	5670
	9:45 a.m.	6000	10	600	86	5160
	9:40 a.m.	6300	8	504	87	5481
I	9:45 a.m.	12200	11	1342	87	10614
	9:05 a.m.	11300	7	791	87	9831
	9:30 a.m.	14400	7	1008	86	12384
	9:42 a.m.	14700	8	1176	87	12789
	9:45 a.m.	12700	8	1016	89	11303
N	9:55 a.m.	5200	13	676	70	3640
	8:55 a.m.	7100	22	1562	66	4686
	8:55 a.m.	5700	9	513	81	4617
	9:25 a.m.	6000	13	780	76	4650
	9:55 a.m.	5800	22	1276	63	3654

TABLE IV
Averages of Hourly Leukocyte Counts of 14 Normal Rabbits

Time of count	Number of determinations averaged	Average total leukocytes per cu. mm.	Average differential percentages and absolute numbers of leukocytes per cu. mm.			
			Neutrophils (amphophils)		Lymphocytes	
			Per cent	Absolute number	Per cent	Absolute number
9:45 a.m. to 10:05 a.m.	12	9600	23	2282	71	6751
10:45 a.m. to 11:05 a.m.	14	9221	24	2303	69	6397
11:45 a.m. to 12:05 p.m.	14	9621	28	2727	65	6237
12:45 p.m. to 1:05 p.m.	14	9914	33	3358	59	5895
1:45 p.m. to 2:05 p.m.	14	9164	35	3242	58	5295
2:45 p.m. to 3:05 p.m.	13	9300	38	3511	54	5148
3:45 p.m. to 4:05 p.m.	11	9800	38	3594	56	5681

the extent of the variations evident from the previous tables dealing with individual animals. When only average determinations are considered, in general the total leukocyte values are fairly constant, the absolute number of lymphocytes slightly decreases and the absolute number of neutrophils (amphophils) slightly increases during the day. Consequently, the differential percentages of lymphocytes and neutrophils (amphophils) slightly decrease and slightly increase, respectively. Percentages and absolute numbers of large mononuclear cells, eosinophils and basophils fluctuate but cannot be considered to exhibit significant trends because these cells comprise only a small fraction of the circulating leukocyte population.

TABLE V
*Increase or Decrease of Afternoon Average Leukocyte Counts, with Reference to Morning Average Leukocyte Counts, in 14 Normal Rabbits**

Rabbit letter	Total leukocytes	Neutrophils (amphophils)	Lymphocytes
A	+3567	+1959	+1664
B	+175	+1022	-910
B	+1700	+2544	-958
C	-1450	+374	-1945
D	+1600	+1853	-280
E	+1750	+1205	+525
F	+870	+510	+129
G	-1191	-205	-900
H	-575	+258	-854
I	-566	+312	-1347
J	+1100	+1187	-159
K	-1458	+1324	-2625
L	-733	+241	-523
M	-2358	+1311	-3643
N	+1075	+1568	-403

* A positive sign indicates that the afternoon average was greater than the morning average by the total difference indicated. A negative sign indicates that the afternoon average was less than the morning average by the total difference indicated.

The increase or decrease of the average of the afternoon total leukocyte counts of individual rabbits, with reference to the average of the morning determinations in each case, is shown in Table V. It is evident that an apparent increase in the average of the afternoon total leukocyte counts was a fairly frequent but not constant finding. These increases or decreases are expressed as percentages of the respective morning averages in each case in Table VI. In this table the afternoon leukocyte fluctuations noted in studies of normal human leukocyte variations by Sabin and co-workers,¹ Shaw,⁸ and Ponder, Saslow and Schweizer,⁶ and ex-

pressed as percentage increases or decreases (with reference to respective morning averages) by Ponder and co-workers,⁶ are compared with similarly expressed percentage increases or decreases in individual rabbits in the present study. Although comparison of human and rabbit experiments may be unjustifiable, it should be emphasized that expression of differences between two variables in terms of percentage of one of these variables may introduce confusion in interpretation of findings. Since biometric analyses seem unjustified in view of the small number of variants in the present study, actual numerical increases or decreases of the individual afternoon averages are listed in Table V.

Further examination of Table V shows that, in the 14 rabbits studied, an apparent afternoon increase in the number of neutrophils (amphophils) appeared to be more frequent than an afternoon rise in the total leukocyte count. An apparent afternoon decrease in the number of lymphocytes seemed to be a fairly frequent, although inconstant, finding. Variations in numbers of blood lymphocytes may, to a large extent, reflect similar changes in lymph flow.⁴

From a practical standpoint, these results seem to indicate that when the hourly leukocyte picture of the rabbit is studied under different experimental conditions, it would seem advisable to de-

TABLE VI
Increase or Decrease of Afternoon Average Total Leukocyte Counts, Expressed as Percentages of Respective Morning Average Leukocyte Counts, in Normal Human Subjects and in 14 Normal Rabbits

Subject	Percentage relationship as found by different authors			
	Normal human subjects			14 normal rabbits
	Ponder <i>et al.</i> ⁶	Shaw ⁸	Sabin <i>et al.</i> ¹	Reifenstein <i>et al.</i>
1	3	26	17	45
2	1	15	15	-13
3	12	-13	14	22
4	4	28	9	30
5	10	30	6	9
6	-24	13	24	-11
7	57	35	..	-6
8	13	20	..	-5
9	..	3	..	11
10	..	15	..	-11
11	-5
12	-18
13	18
14a*	3
14b*	29

* 14a and 14b represent the same rabbit (B) counted on two occasions.

termine the general normal leukocyte trends of each animal and then maintain the same period of the day for counting at hourly intervals under experimental conditions, as well as to establish similar control of other possible sources of error in the determinations.

SUMMARY

Hourly total and differential leukocyte studies of 14 normal rabbits are presented.

Individual examinations show considerable variation; average counts exhibit tendencies for the total numbers of leukocytes to vary little, and for the percentages and absolute numbers of lymphocytes and neutrophils (amphophils) to decrease and increase slightly, respectively, during the day.

An apparent afternoon increase in the number of neutrophils (amphophils) is a more frequent finding than an apparent afternoon increase in the total leukocyte count.

In studying the hourly leukocyte picture of rabbits under different experimental conditions, it would seem advisable to determine general normal leukocyte trends for each animal and then establish the same periods of the day for making counts under experimental conditions.

REFERENCES

1. Sabin, F. R.; Cunningham, R. S.; Doan, C. A., and Kindwall, J. A. The normal rhythm of the white blood cells. *Bull. Johns Hopkins Hosp.*, 1925, 37, 14-67.
2. Bushnell, L. D., and Bangs, Edna F. A study of the variation in number of blood cells of normal rabbits. *J. Infect. Dis.*, 1926, 39, 291-301.
3. Cheng, S. C. Leucocyte counts in rabbits: observations on the influence of various physiological factors and pathological conditions. *Am. J. Hyg.*, 1930, 11, 449-533.
4. Garrey, W. E., and Bryan, W. Ray. Variations in white blood cell counts. *Physiol. Rev.*, 1935, 15, 597-638.
5. Bryan, W. Ray; Chastain, L. L., and Garrey, W. E. Errors of routine analysis in the counting of leucocytes. *Am. J. Physiol.*, 1935, 113, 416-429.
6. Ponder, Eric; Saslow, George, and Schweizer, Malvina. On variations in the white-cell count of man. *Quart. J. Exper. Physiol.*, 1931, 21, 21-35.

7. Pearce, Louise, and Casey, Albert E. Studies in the blood cytology of the rabbit. I. Blood counts in normal rabbits. *J. Exper. Med.*, 1930, 51, 83-97.
8. Shaw, A. F. Bernard. The diurnal tides of the leucocytes of man. *J. Path. & Bact.*, 1927, 30, 1-19.
9. Zirm, Konrad L., and Bauermeister, Wolf. Über physiologische Tageschwankungen der Leukocyten. *Ztschr. f. klin. Med.*, 1933, 125, 282-293.
10. Pearce, Louise. Personal communication.